December 14, 1893.

Sir JOHN EVANS, D.C.L., LL.D., Treasurer and Vice-President, followed by Professor J. S. BURDON SANDERSON and Sir G. M. HUMPHRY, in the Chair.

A List of the Presents received was laid on the table, and thanks ordered for them.

The Right Hon. James Bryce, a Member of Her Majesty's Most Honourable Privy Council, whose certificate had been suspended, as required by the Statutes, was balloted for and elected a Fellow of the Society.

The following Papers were read:—

I. "On the Constitution and Mode of Formation of Food Vacuoles in Infusoria, as illustrated by the History of the Processes of Digestion in *Carchesium polypinum*." By MARION GREENWOOD, Girton College, Cambridge. Communicated by J. N. LANGLEY, F.R.S. Received October 28, 1893.

(Abstract.)

Since the time that Ehrenberg first formulated his celebrated "polygastric" theory, there have been few writers on the Infusoria who have not confirmed his observations while combating the interpretation of them given by him. For it was shown long ago that the "Magenzellen" of Ehrenberg are spherical food masses, and these, circulating with varying rapidity in the "endoplasm," are so striking optically in most Infusoria that the literature of this group abounds in descriptions of or references to them. There is, however, a lack of any details which would throw light on the precise mode of origin of these ingesta, and yet this point has interest, for, while relatively large food masses are swallowed by certain of the ciliate Infusoria, very many forms are adapted only for the inception of minute solid particles, which seem far removed from the relatively large masses so noticeable within the animals.

An extreme case of this disparity between the size of ingested particles and the size of food masses circulating within the body is found in almost any member of the Vorticellidæ; the form I have chosen for examination is *Carchesium polypinum*, which grows in pedicellate clusters, each polype being mounted on a highly retractile

stalk, and being made up of relatively transparent cell substance. This animal shows such marked voracity upon occasion that I have counted 100 food masses within its substance; at the same time it is only minute particles which are acceptable when offered for ingestion. The shaping of the one form of matter from the other, then, promises to be a process of no small interest, and actual observation has made me think that it is more than merely interesting, that it may be regarded rather as *the striking illustration of a process often masked in other Protozoa, but fundamental in nature.*

I have fed *Carchesium* with nutritious and innutritious particles, with milk, with bacteria, with such flocculent precipitates as are thrown down by the interaction of ditch water and alizarin sulphate or congo-red, and with pigment grains—carmine, Indian ink, or ultramarine blue. All these are ingested readily, but the particles which perhaps serve best to illustrate the striking events of digestion are the finely-divided granules of proteid which form a precipitate when diluted white of egg is coagulated by heat. When the white of fresh eggs is treated thus, the heat precipitate is generally abundant; a very scanty coagulum may form, however, when the eggs from which the diluted fluid is prepared are stale. It is known that any marked alkalinity hinders effective coagulation of albumen and that albumoses and peptones do appear in stale white of egg; I think it fair, then, to suppose that *Carchesium* ingesting the abundant coagulation precipitate is ingesting minute irregular fragments of nutritious matter suspended in a dilute solution of salts, and that in administering the merely opalescent fluid a less obvious but possibly important substance is supplied—soluble food. All ingested particles, whatever their nature, pass from the exterior by a slightly sinuous ciliated pharyngeal tube which leads inwards from the wide mouth, and, spacious itself at first, narrows, to end internally in a small dilated sac, the œsophagus.* An anal ridge runs at right angles to the long axis of the polype at the junction of the outer and middle thirds of the pharynx, and from this ridge all effete matters are ejected, but food particles are gathered by the oral and pharyngeal cilia into the œsophageal sac, and, mixed in varying proportions with the fluid medium in which the animal is living, start from its most internal point on their intracellular career as a *vacuole of ingestion.*

Each vacuole of ingestion thus discharged by some obscure impulse performs a movement of *progression;* it passes with a steady gliding motion towards the basal attachment of the polype, coming to rest at some point along the concavity of the band-like nucleus. A period of *quiescence* follows and persists in healthy specimens for some seconds, and at its end the vacuolar contents, which up to this

* I have adopted the terminology of R. Greef ('Archiv für Naturgesch.,' Wiegmann, vol. 37, 1871).

point have been apparently in the condition in which they left the œsophagus, are rearranged in a very remarkable fashion. *The solid particles, it may be of proteid, of pigment, or bacteria, are gathered to a cluster, with a rapid centripetal movement; those which are more peripheral leave the boundaries of the vacuole, and a composite solid mass lies in clear fluid surroundings.* The outline of constituents so strikingly individual as are bacteria or the fat globules of milk may still be made out in the cohering cluster, but Brownian movement is ended, as are the "proper" movements of any small organisms which may be present, and further change is not in the direction of freedom, but makes the union closer; it tends to perfect the homogeneity of the composite solid. To this rearrangement of matter I apply the term "*aggregation*,"* for the obvious feature of the act is the clustering of particles of matter which were scattered before. It is most clearly demonstrable in vacuoles which contain but few minute particles suspended in a relatively large amount of water; it is masked when the solid matter preponderates or is less finely divided. No distinct relation can be traced, however, between the chemical character of the ingested matter and energy of aggregation, for nutritious and innutritious particles are moved with equal vigour and show equally little tendency to immediate subsequent separation. I might enumerate no inconsiderable number of variations of this process, some dependent on peculiarities of the ingesta dealt with (the presence of organic matter in solution, the rare enclosure of filamentous bacteria), some related rather to changes in the condition of *Carchesium* (abnormally eager ingestion or exceptionally lethargic action), but through all the modifications I have observed the salient characteristic of aggregation of solids and synchronous separation of fluid may be traced, and in face of each, the question arises, "*What force effects this movement and insures this redistribution of matter?*"

In answer to this question, three hypotheses may be considered:—

1. It may be supposed that as particulate proteid matter if pressed together with some force tends to form masses which cohere after the pressure is removed, so in the vacuole of ingestion the approximately symmetrical discharge of small jets of fluid from the surrounding protoplasm carries solid particles centripetally, and that, after displacement of the water between them, they cohere. It is noticeable, however, that grains of Indian ink may be united firmly in aggregation and discharged as a solid mass, that relatively large granules (such as the fat drops of milk) are inseparable after the first marked centripetal shifting,† and that nothing is more striking than

* I use this term with some reluctance in face of the fact that it was applied by Darwin many years ago ('Insectivorous Plants') to an entirely different process in the cells of the tentacles of *Drosera*.

† It will be gathered from what has been said above that only when these

the firm unbroken edge of a composite solid which abuts on the fluid of the vacuole in which it was formed. On these and other grounds this hypothesis appears to me inadequate, and I would lay but little stress upon it.

2. Again, it may be believed that, as in plasmolysed vegetable cells, the primordial utricle shrinks centripetally, gathering up in its retreat all granules which lie within or throughout its substance, so here *a highly elastic pellicle, living, or the product of secretion,* is set free from the walls of the vacuole and contracts rapidly, gathering within its lessening circumference all the solid particles which were suspended freely before. The unbroken line, which from the moment of *aggregation* marks off the clustered particles from the fluid in which they lie, does indeed suggest the presence of an enclosing film, but other experimental facts are clearly out of harmony with an hypothesis which postulates its existence. Thus the final cohesion of aggregated particles is at least as perfect in the centre of the composite mass as round its circumference; when by certain changes which may follow aggregation any particles are set free slowly, it is from the outside of the food mass only, and ejection from the body never means disintegration of the contents of an excretory vacuole, but is rather the freeing of a resistent solid. Further, there is, rarely, a want of synchronism in the aggregation of the particles in a vacuole; Brownian movement may persist for a time towards the end of the vacuole, when the majority of the granules present are quiescent; in other rare cases the aggregated mass clings to the cell substance from which it is (for the most part) separated by fluid, by slender threads of almost invisible, possibly mucilaginous, substance, these threads breaking presently and being dragged into the central solid; and it is usual to find that actual measurement of a food mass demonstrates the persistence of secondary shrinking after *aggregation* is over. I am inclined then to think that a third hypothesis meets the case more fully than either of those just mentioned, and to suggest—

3. *That the solid particles which undergo change of position in aggregation are dragged together by the comparatively rapid retraction of some substance contained in the vacuole; this substance is probably viscous.*

Such an hypothesis does not, however, offer any description of the mechanism of retraction, and the nature of this mechanism is certainly obscure. But I may point out that there are some undoubted resemblances between this rearrangement of substance and the phenomena which are grouped together as "clotting actions." In all perfected clots we have to recognise the interaction of two bodies or it may be the reconstitution of one body removed from the seat of most vigorous metabolic change—the cell, and a separation of solid

relatively large particles are present scantily in a vacuole do they move markedly in *aggregation.*

matter and subsequent shrinking, both varying in character and extent, are common accompaniments of the fundamental chemical reaction of clotting.

In *Carchesium*, then, it may be that we meet with a modification of the process, that there is an intravacuolar discharge of matter which clots, shrinking rapidly, and not with the slow change of casein or fibrin, and that it, entangling any solid particles which are present, brings about the spasm of *aggregation*. The substance is, indeed, not demonstrable usually by staining or other form of micro-chemistry, but delicate indicators of the presence of acid introduced into a digestive vacuole indicate that the vacuolar fluid begins to have an acid reaction about the time when *aggregation* is perfected. It is conceivable, then, that an access of acid fluid at this point may help the effective retraction of the clot, or even its first formation.

When by the process of aggregation spherical ingesta have been welded together in the substance of *Carchesium* from digestible or indigestible particles, they journey through the "endoplasm" in a fairly constant fashion, but for a variable time. Occasionally they are stored for hours after loss of the fluid of those vacuoles in which *aggregation* occurred; at times the digestion of nutritious matter follows the preliminary clustering with no marked pause. All nutritive ingesta present are not of necessity digested synchronously; indeed, there is sometimes apparent caprice in solution, but certain features of the process are invariable whenever its onset occurs.

Thus, as in *Amœba*, solution is effected in a *fluid medium*. The stored up food masses of *Carchesium*, when they have lost their fluid surroundings, have reached the extreme point of density and shrinkage; solution implies swelling, transparency, and *re-formation of a vacuole* if it has not persisted. Digestion may take place at any point throughout a relatively large part of the central substance of *Carchesium*, but complete solution is extremely rare, and innutritious remains travel with varying rapidity towards the anal ridge from which they are discharged. Thus they pass into the pharynx, to be swept to the exterior eventually by ciliary currents. It may be said that, other things being equal, the intracellular sojourn of ingesta tends to vary directly with their digestibility; thus clusters of aggregated particles in which such bodies as carmine or Indian ink preponderate or stand alone have a relatively short time of enclosure; the fluid of the vacuoles in which they are formed often disappears quickly, and there is but rarely (in the case of unmixed innutritious matter) that re-formation of fluid which is so nearly concerned in the solution of true food stuffs.

I have spoken hitherto with some vagueness of the duration of

successive events in the digestive process in *Carchesium*, and, indeed, the variability of many of them is marked. Changing conditions of the animal, on the one hand (and some of these changes cannot easily be controlled by the experimenter), and alteration in surroundings which may be bound up with temperature, aeration, illumination, or food, on the other, tend to bring out the elasticity of some of the periods which I have distinguished. But this elasticity has limits, nor does it characterise equally all the phases in the digestive cycle. We find that the total time of enclosure of innutritious matter may be as short as 30 minutes, that nutritious substances have a minimum (recorded) sojourn of 1 hour to $1\frac{1}{2}$ hour, and, on the other hand, that the time of enclosure may be prolonged to 30 hours. This great variation is found on examination to belong to that period in the history of ingesta in which they are stored, inert and destitute of fluid surroundings. The interval which separates successive acts of ingestion in any one series varies from 30 sec. to 65 sec., and is commonly 40 sec.; the duration of the movement of *progression* varies inversely (roughly speaking) with the duration of the phase of *quiescence*. Thus, progression may occupy $5\frac{1}{5}$ sec. or $14\frac{2}{5}$ sec., but is often $10\frac{2}{5}$ sec.; quiescence, with a usual duration of 9 sec., may be shortened to 5 sec., or lengthened to $25\frac{2}{5}$ sec. *Aggregation* is, as a rule, instantaneous in vigorous animals, but $\frac{2}{5}$ sec. or even $\frac{4}{5}$ sec. have passed between the onset and completion of the movement. The act of *solution* is more variable; I have seen very far reaching digestive change in 50 min., but that variation should be more striking than constancy is hardly surprising in face of the unlike nature of possibly digestible matter. Lastly, as I have said, the stage of *storage* may be omitted; in this case digestion succeeds *aggregation* at once, the fluid of the digestive vacuole increasing in amount and (presumably) changing in composition; on the other hand, ingesta may be stored for 22 hours before they are attacked by the true digestive secretion.

I have said above there seem to me to be grounds for regarding the aggregation of ingested particles in this complete and vigorous manner as a fundamental process in Protozoan digestion. Striking as the phenomenon is in *Carchesium*, the actual displacement of matter which it involves is, of course, small, and effective demonstration is possible chiefly because of the great transparency of the acting cell substance, and because the food is naturally, or may be kept artificially, in a state of minute division. Even in *Carchesium*, however, the marked retractility of the hyaline stalk of each polype often hinders observation; and, when it is remembered that so many Protozoa are vigorously motile, or relatively opaque, or deal (as do the Rhizopods) with comparatively massive food, it is hardly wonderful that a secretion of matter which is (by virtue of its own

characters) practically invisible, has occurred without exciting especial comment. R. Greef, in the paper to which I have referred, gives what I take to be a very brief description of the process of aggregation in *Epistylis flavicans*, and, looking back at observations made on the digestive processes in *Amœba* some time ago, I feel that many which were puzzling then are in harmony with the experimental results recorded above. Among these I may instance the very sudden quiescence after enclosure of such small organisms as monads, the firm union of unlike ingesta which were by chance enclosed together and so came to be in a common vacuole, and the cohesion after ingestion of particles of carmine or Indian ink.

And if further work should replace these scattered points of likeness by fuller, harmonious observations, then I think that the process of aggregation, owing the interest which it possesses, not to the obvious movement of particles, but to the more hidden mechanism which carries out the movement, may be allowed to have some such functional value as that indicated in *Carchesium* by the constancy of its duration and the constancy of its occurrence, whatever the chemical nature of the foreign particles involved. It may perhaps rank as an expression of what has been lacking among the Protozoa—what is clear enough among Cœlenterata, with their well-defined, unicellular glands—as an expression of *obscure histological change bound up with the digestion of food, or more nearly with its preparation for digestion.*

II. "The Action of Light on Bacteria. III." By H. Marshall Ward, D.Sc., F.R.S., Professor of Botany, Royal Indian Engineering College, Coopers Hill. Received December 14, 1893.

(Abstract.)

Several observers, notably Arloing, Janowsky, Geisler, and Chemelewsky, have tried to determine which rays of the spectrum are chiefly concerned in the destruction of bacteria, but all attempts hitherto have been made by placing separate tubes of broth, gelatine, or potato cultures in the various regions of the spectrum, and judging of the relative rates of growth by the periods in which turbidity is apparent, or by the sizes of the respective growths on solid cultures, and their conclusions vary considerably.

The author has succeeded in obtaining photographic records by throwing the spectrum on an agar film evenly charged with the spores or bacilli to be investigated, and then observing the behaviour of the illuminated regions after incubation.

Various species have been employed, *Bacillus anthracis*, *B. sub-*